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**"Success usually comes to those who are too busy to be looking for it"**

# CSIR NET – Life Science

# Unit 1: Biochemistry

## 11 Protein Stability & Kinetics

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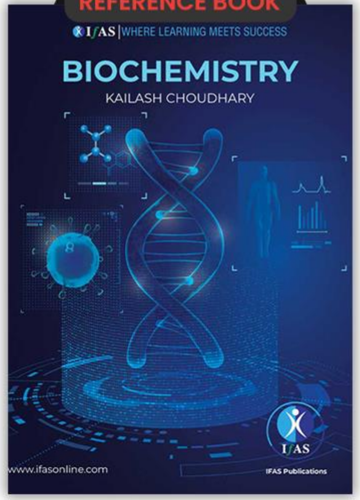


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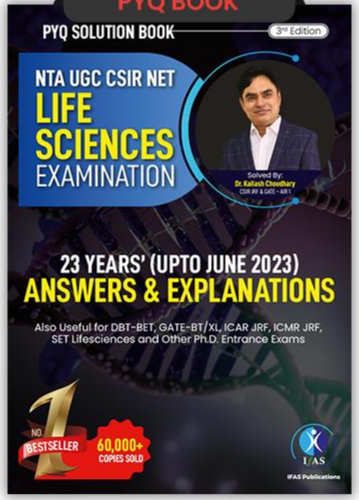


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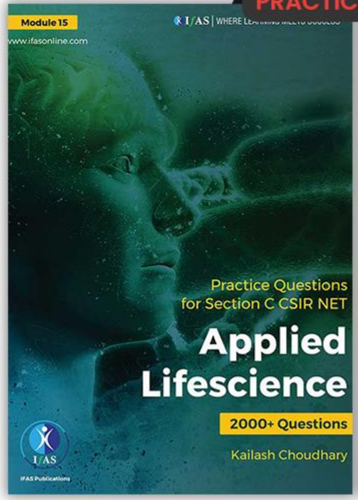
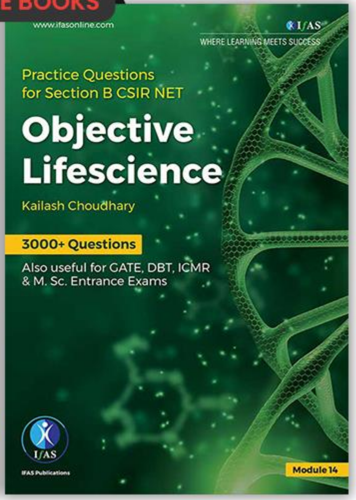


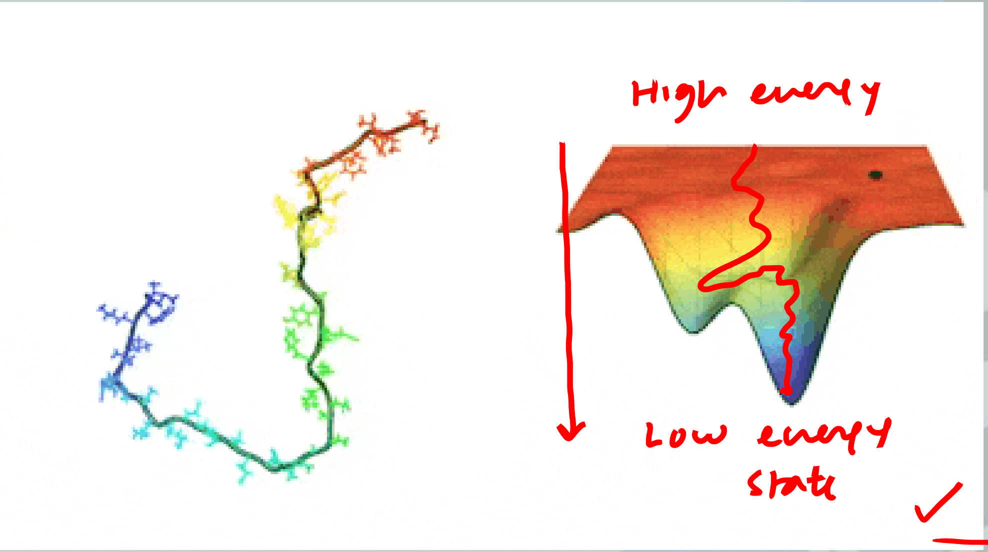



# Points to be covered in this Lecture

- Protein folding
- Stability and solubility of Protein
- Rate of reaction
- Order of reaction
- What are Biocatalyst
- Factors affecting enzyme activity

## How do protein fold perfectly each time?

$2^{\circ} \longrightarrow 3^{\circ}$




**Driving force: Hydrophobic Interactions**

During folding hydrophobic amino acids tend to move towards the inside of the protein structure, away from the aqueous cellular environment, while hydrophilic residues are more likely to be exposed on the surface.

Decrease in entropy of protein (solute)

**Increase in entropy of water (solvent)**

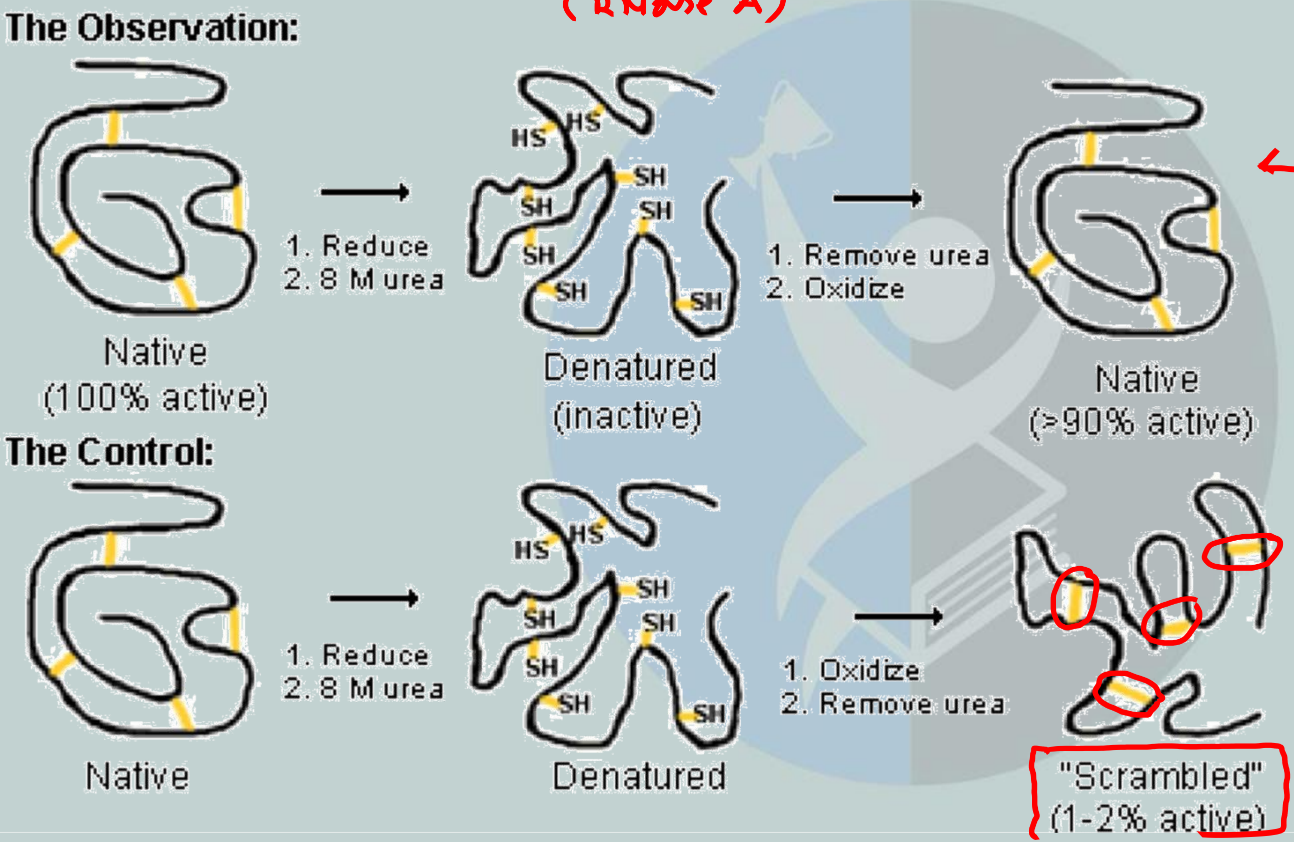
Increase in entropy of solution

**Protein always adopt form which has least free energy**

## Reversible Denaturation of Ribonuclease A: Christian Anfinsen

(RNase A)

(1) Protein fold
(2) Disulphide bonds may form.



## Levinthal's paradox

$\phi^1 \; \phi^2 \; \psi^3 \quad \phi \; \psi \; \phi \; \psi^{100} \; \psi$

If a protein with 100 amino acids, it would have 99 peptide bonds and 198 considerations for ϕ and ψ angles.

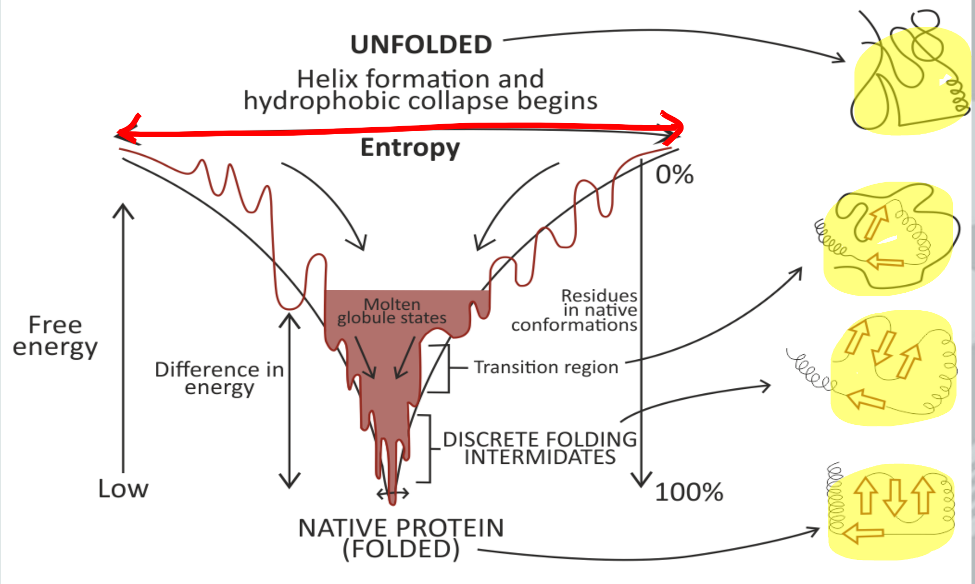
If each of these had only three conformations, that would result in $3^{198}$ different possible folding.

$3 \times 3 \times 3 \ldots\ldots\ldots = \boxed{\ldots\ldots\ldots}$

Levinthal suggested, *proteins must fold by some sort of ordered pathway or set of pathways in which the approach to the native state is accompanied by sharply increasing conformational stability (decreasing free energy).*

## Energy Landscape Theory (Folding Funnel):

*Polypeptides fold via a series of conformational adjustments that reduce their free energy and entropy until the native state is reached*




• unfolded (molten Globule)
many 2° str & no 3° str
Free energy = ↑, H = ↑, S = ↑
↓
↓ Intermediate states
↓
Native folded state (3°)

G = ↓
H = ↓
S = ↓

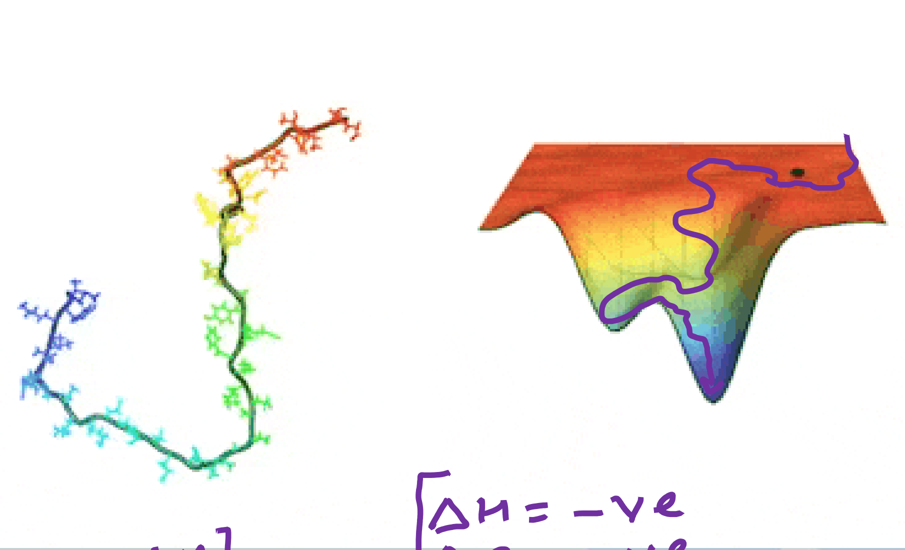
$\Delta G = -ve$
$\Delta H = -ve$
$\Delta S = -ve$

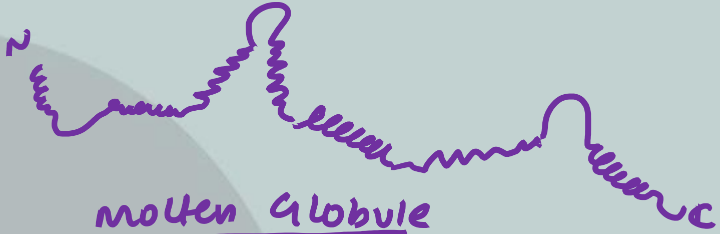
$T < \frac{\Delta H}{\Delta S}$

**Single largest contribution to the stability of a folded protein is ΔS (solvent) for the nonpolar residues.**

water entropy = ↑

# Thermodynamics of protein folding






Molten Globule

$\downarrow$

Native state

$$T_m = \frac{\Delta H}{\Delta S}$$

$\Delta H = -ve$
$\Delta S = -ve$
$\Delta G = -ve$

$$T < \frac{\Delta H}{\Delta S}$$

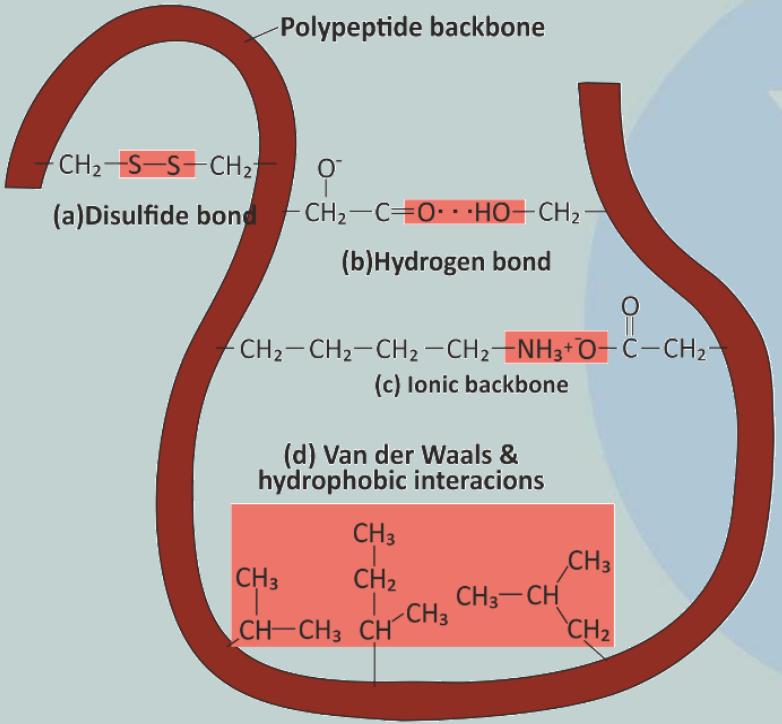
$$UF \rightleftharpoons \text{folded}$$

$$K_{eq} = \frac{[F]}{[UF]}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

$$\Delta G^\circ = -2.303\, RT \log K_{eq}$$

# PROTEIN STABILITY



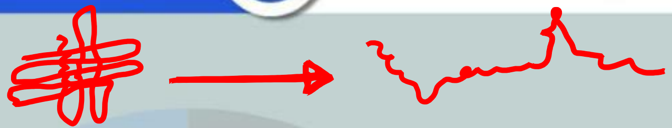

- **Primary Structure:** Covalent bonds (Peptide bond)
- **Secondary structure:** Hydrogen bonds
- **Tertiary structure:** Hydrophobic interactions

3D structure is stabilized by
- Non-covalent interactions
  - Hydrophobic
  - hydrogen bond
  - electrostatic
  - vandewalls
- Disulphide bonds


[3D structure]

- X-Ray crystallography
- 2D-NMR spectroscopy
- Cryoelectron microscopy

## Conditions for denaturation



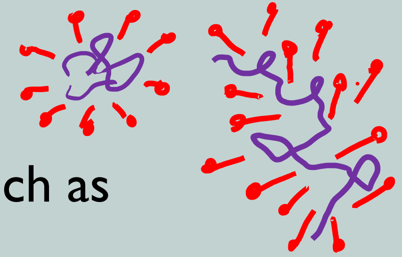
**pH variations:** alter the ionization states of amino acid side chain

Asp, Glu
Lys, Arg
His

pH = ↓ pH = ↑

**Detergents**: hydrophobically associate with the nonpolar residues

↳ SDS (−), CTAB (+), Triton-X (uncharged)

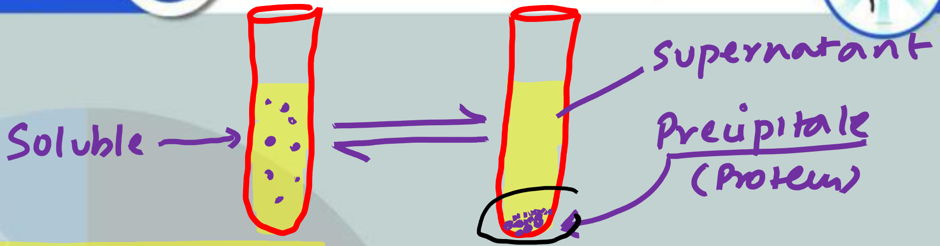


**High concentrations of water-soluble organic substances** such as aliphatic alcohols interfere with the hydrophobic forces

- Acetone ↑
- Butanol ↑
- Petroleum ether ↑

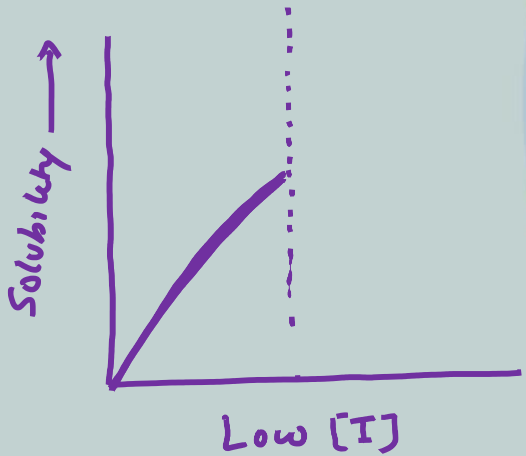
# SOLUBILITIES OF PROTEINS

## A. *Effects of Salt Concentrations*



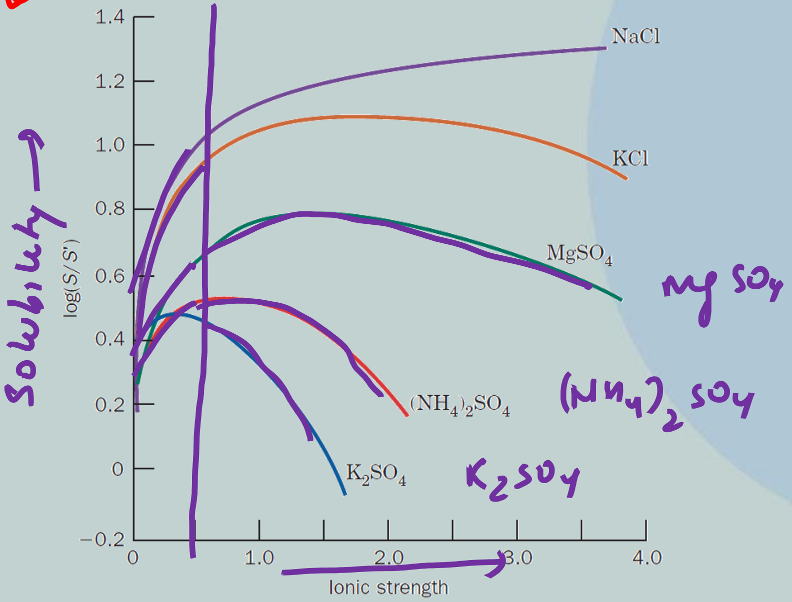

**Salting in:** *The solubility of a protein at low ionic strength generally increases with the salt concentration*

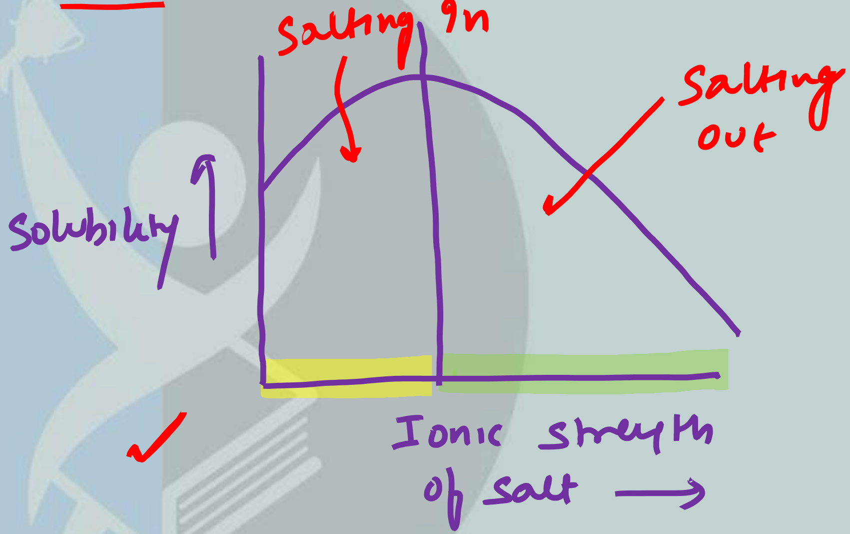
$$I = \frac{1}{2} \sum c_n \cdot z_n^2$$



## Salting out:

- The solvent's activity is decreased. protein water
- Solute–solute interactions become stronger than solute-solvent interactions and the solute precipitates.
  protein - protein
- Ammonium sulfate

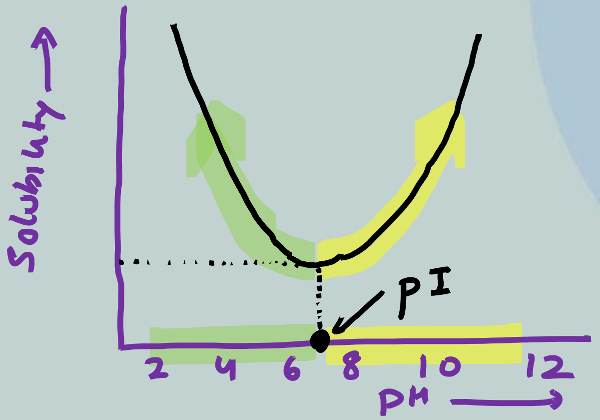


## B. Effects of pH

Iso-electric precipitation: Solubility is minimum when surrounding pH is equal to pI of protein

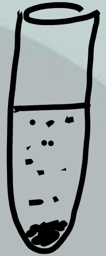
| | Charge | Solubility |
|---|---|---|
| pH = pI | 0 | minimum |
| pH < pI | + | ↑ |
| pH > pI | − | ↑ |



## C. Effects of Organic Solvents

**Precipitant :** Low dielectric constant

Acetone and ethanol



**Solvent:** High dielectric constant

Dimethyl sulfoxide (DMSO) or *N*,*N*-dimethylformamide (DMF)

Glycerol

→ Hydration of protein

→ solubility = ↑

# Chemical Kinetics

$\boxed{\text{Spontaneous}}$

- Standard conditions
- Physiological condition

$$A \xrightarrow[\Delta G = -ve]{\Delta G^{\circ} = -ve} B$$

Rate = ? No idea

fast or slow

'Kinetics'

# Reaction Kinetics

## 1. Rate of a Reaction or speed or velocity

It is defined as the change in concentration of a reactant or product per unit time. It is usually expressed in terms of molarity per second (M/s).

$$A \rightarrow B$$

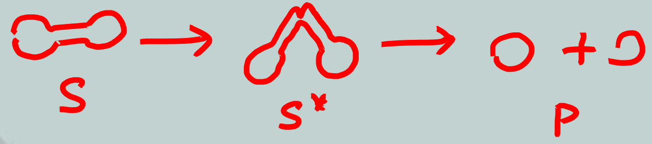
Reactant M    Product M

$$\text{Rate} = \frac{-\Delta[A]}{\Delta t} = \frac{\Delta[B]}{\Delta t} \leftarrow \frac{M}{sec}$$

$$M = \frac{mol}{Lt}$$

$M\ sec^{-1}$    $mol\ lt^{-1}\ sec^{-1}$

# Factors affecting Rate of a Reaction



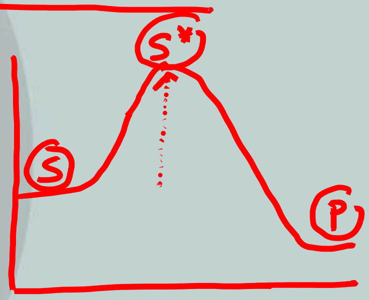

## 1. Concentration of Reactants:

Increasing the concentration of reactants increases the rate of reaction.

## 2. Temperature

$Q_{10} = 2$ Rate doubles with change of every 10°C

Increase in temperature increases the rate of reaction.




| | Rate |
|---|---|
| 5° | 1 |
| 15° | 2 |
| 25° | 4 |
| 35° | 8 |
| 45° | 16 |

$Q_{10} = 2$

## 3. Surface Area of Reactants:

large size Rate = ↓ $\frac{SA}{Volume} = \downarrow$

small size = Rate = ↑ $\frac{SA}{Volume} = \uparrow$

## 4. Light /Radiations:

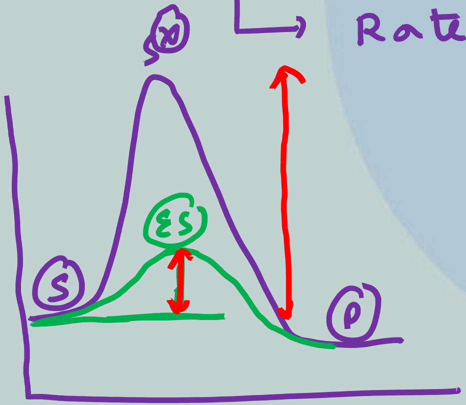
visible — X-Rays
UV — microwaves

$\left.\begin{array}{l}\text{visible, UV} \\ \text{X-Rays, microwaves}\end{array}\right\} \rightarrow$ source of energy

$S \longrightarrow S^{*} \longrightarrow$ Product

## 5. Presence of a Catalyst:

→ Lower activation energy
→ Rate of rxn increases



# 2. Molecularity of a reaction

Number of molecules reacting in an elementary step.

A $\longrightarrow$ Product unimolecular

Glucose-6-P $\longleftrightarrow$ Fructose-6-P

A + B $\longrightarrow$ Product Bimolecular

Glucose + ATP Glucose-6-P + ADP

A + B + C $\longrightarrow$ Product Termolecular

PEP + ADP + iP Pyruvate + ATP

# 3. Order of reaction:

How the reaction rate is influenced by the **concentration of the reactants** under a given set of conditions.

It is sum of the powers to which all reactant concentrations are raised in the rate law equation.

$$mA + nB \longrightarrow \text{Product}$$

AB

rate $\propto [A]^m [B]^n$

rate $= K [A]^m [B]^n$

The rate law for a reaction can be expressed generally as $\text{rate}=k[A]^m[B]^n$

• m and n are the orders of the reaction with respect to reactants A and B, respectively. The overall order of the reaction is m+n

# First order reaction:

- The rate of reaction is directly proportional to the concentration of one reactant.
- A common example is radioactive decay.

$$A \longrightarrow \text{Product}$$

$$\frac{dc}{dt} \propto -[A] = [P]$$

$$\frac{dc}{dt} = -K[A] = K[P]$$

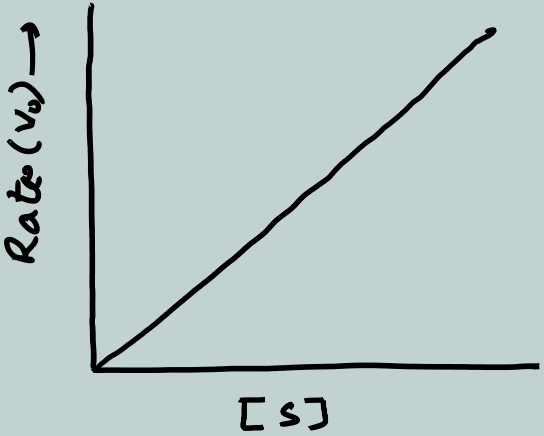
## Unit of first order rate constant:

$$\frac{M}{S} = K \cdot M$$

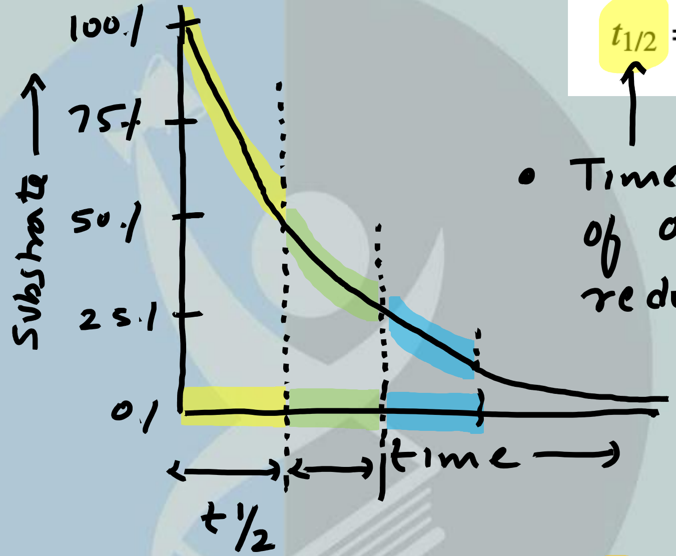
$$K = \frac{\cancel{M}}{S} \cdot \frac{1}{\cancel{M}}$$

$$K = 1/s = s^{-1} \ (sec^{-1})$$

## Rate v/s Substrate




## Concentration v/s time




$$t_{1/2} = \frac{\ln 2}{k} \approx \frac{0.693}{k}$$

- Time in which concⁿ of original substrate reduces to half
- $T_{1/2}$ is constant

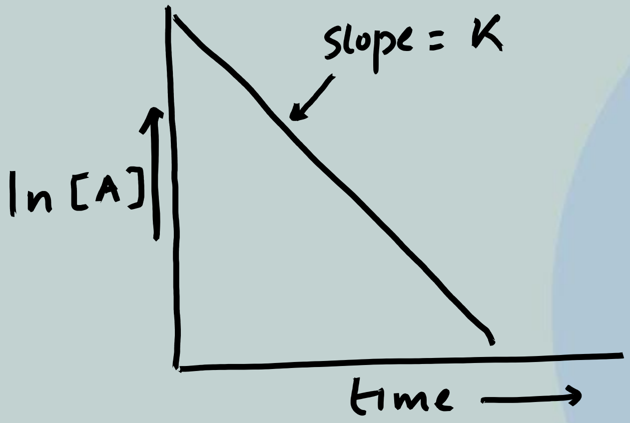
$T_{1/2} \propto \frac{1}{K}$  $T_{1/2} = \frac{\ln 2}{K}$

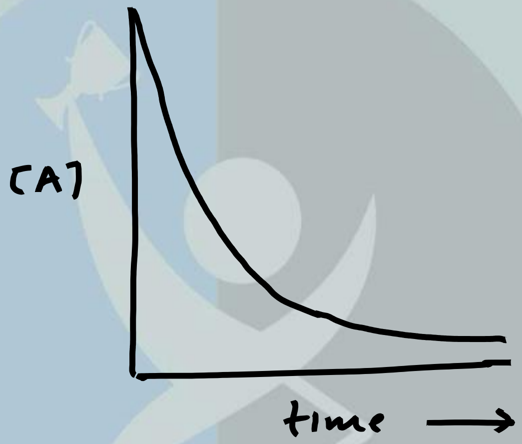
$100 \xrightarrow{1} 50\% \xrightarrow{2} 25\% \xrightarrow{3} 12.5 \xrightarrow{4} 6.25$

$0\% \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow \leftarrow$

Infinite time

## For straight line plot ln[A] with time



## Apply your mind:

If half life of Radioisotope is 10 min, what would be the concentration of radio isotope left after 30 minutes?

(1) 12.5 %
(2) 25 %
(3) 50 %
(4) 75 %

$\frac{30 \text{ min}}{10 \text{ min}} = 3 \leftarrow$ Half life.

left

$100 \xrightarrow{1} 50 \xrightarrow{2} 25 \xrightarrow{3} 12.5$

Decay 0 50% 75% 87.5%

100-100 = 0 100-50 = 50 100-25 = 75 100-12.5 = 87.5

## Apply your mind:

If half life of radioisotope is 1 day, in what duration its concentration will reach to zero?

(1) 1 min

(2) 1 day

(3) 1 year

(4) Infinite

## Second order reaction:

| | | | | | | |
|---|---|---|---|---|---|---|
| [S] | 1 | 2 | 3 | 4 | 5 | 6 |
| $V_0$ | 1 | 4 | 9 | 16 | 25 | 36 |

← second order

The rate of reaction is proportional to the square of the concentration of one reactant

$[A]^1 + [A]^1 \longrightarrow$ Product

SSDNA + SSDNA $\longrightarrow$ DS DNA

$A + B \longrightarrow$ Product

$\frac{dc}{dt} = K [A]^1 [B^1]$

order = 1+1 = 2

$\frac{dc}{dt} \propto [A]^2$

$\frac{dc}{dt} = K \cdot [A]^2$

order = 2

mole$^{-1}$ lt sec$^{-1}$

## Unit of second order rate constant:

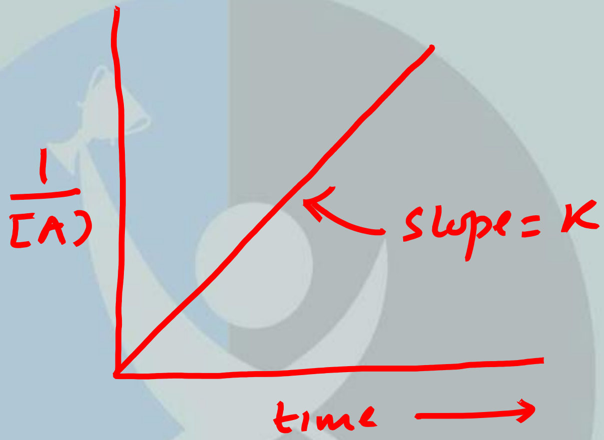
$\frac{M}{Sec} = K \cdot M \cdot M$

$K = \frac{\cancel{M}}{sec} \cdot \frac{1}{\cancel{M}} \cdot \frac{1}{M}$

$K = \frac{1}{M} \cdot \frac{1}{sec}$ or $M^{-1} sec^{-1}$

## Concentration v/s time


For straight line plot 1/[A] with time



## Zero order reaction:

The rate does not vary with the increase or decrease in the concentration of the reactants

$$A + B \longrightarrow \text{Product}$$

Rate = k ✓

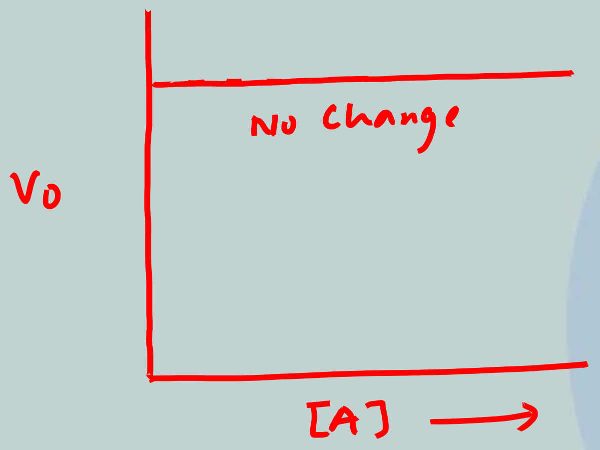
$\boxed{K}$

- I order : $sec^{-1}$
- II order : $M^{-1} sec^{-1}$
- Zero order : $M^{1} sec^{-1}$

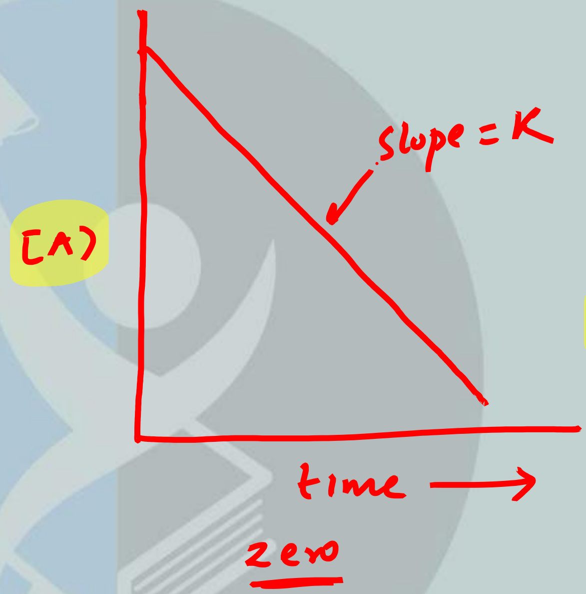
Imp

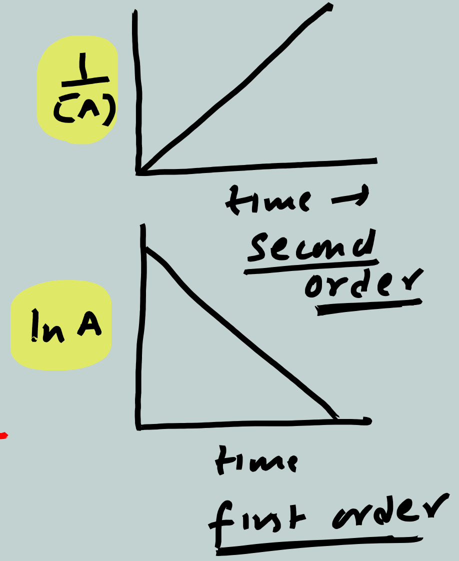
**Unit of zero order rate constant:** $\frac{M}{sec} = M^{1} sec^{-1}$

## Velocity versus substrate



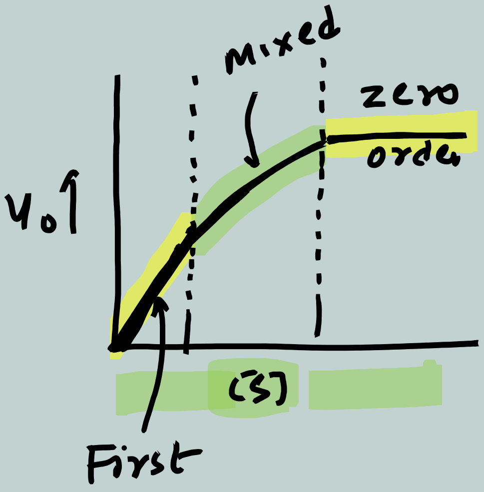

## Concentration versus time

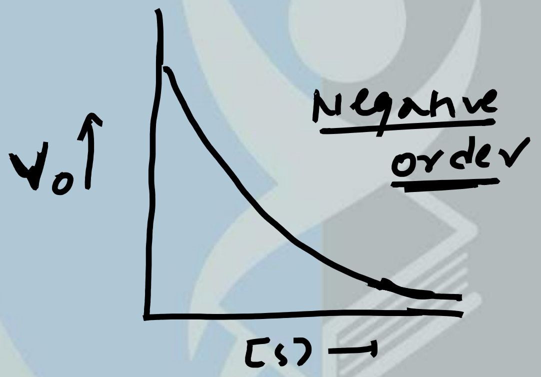


## Fractional (mixed order) and Negative Orders:

Sometimes, the order of a reaction with respect to a reactant can be fractional or even negative, depending on the reaction mechanism.




$V_o$ = observed velocity

# Enzymes

Bio - catalyst

in - yeast

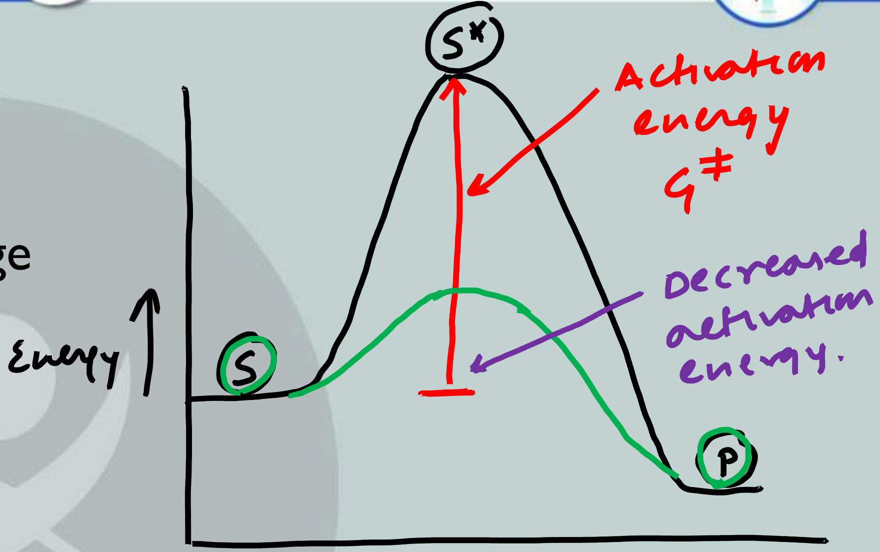
catalyst

Accelerate rate of reaction

## What are catalyst?

- Participate in reaction
- Not consumed or any permanent change
- Required in very small quantities.
- Lowers the activation energy
- Enhance the rate of reactions
- Do not change the equilibrium.
- Do not influence free energy change.

$\Delta G^{0} = -2.303\ RT \log K_{eq}$




$\Delta G = -ve$

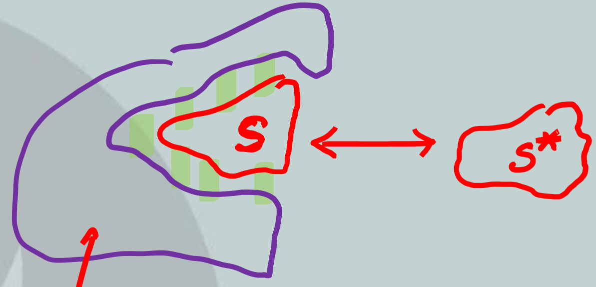
(No change)

- catalyst donot decide direction of reaction
- Allow equilibrium to get achieved in small time.

## Enzymes/Biocatalyst: Organic in nature

99% less than 1%

- Proteins or RNA
- Substrate specific, stereo-specific
- Enhance rate of reaction by forming weak interactions with substrates.
- Work at optimum pH and temperature




3D Str ($3^\circ$ or $4^\circ$)
(Active)

# Active site of enzyme

• atleast 100 aa

*The active site is a 3-D cleft formed by R-groups of amino acids that come from different parts of the amino acid sequence.*

Framework


Active site

*The active site takes up a relatively small part of the total volume of an enzyme.*

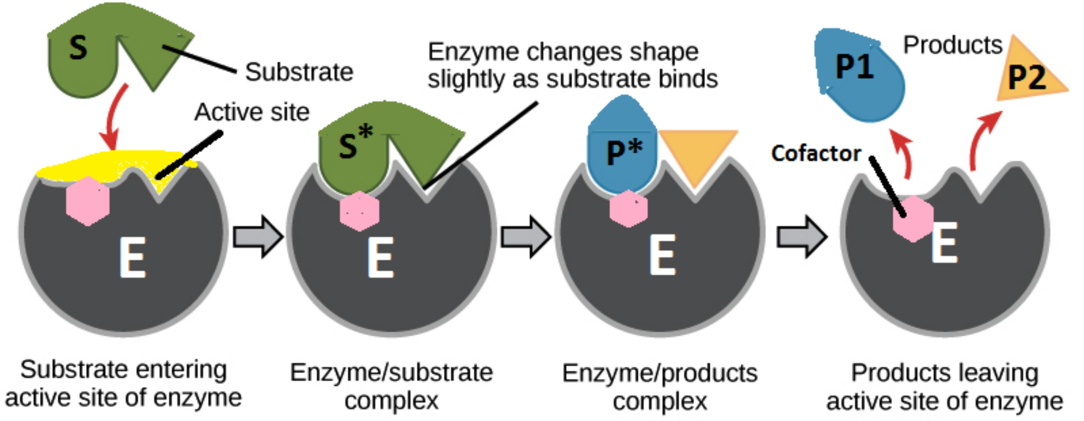



**Figure 3: Active site of Enzyme and Enzyme catalysis**

Active site

- Substrate(s) binding site
- Co-factor(s) binding site
- catalytic site.

More than 100 aa

## Enzyme Specificity

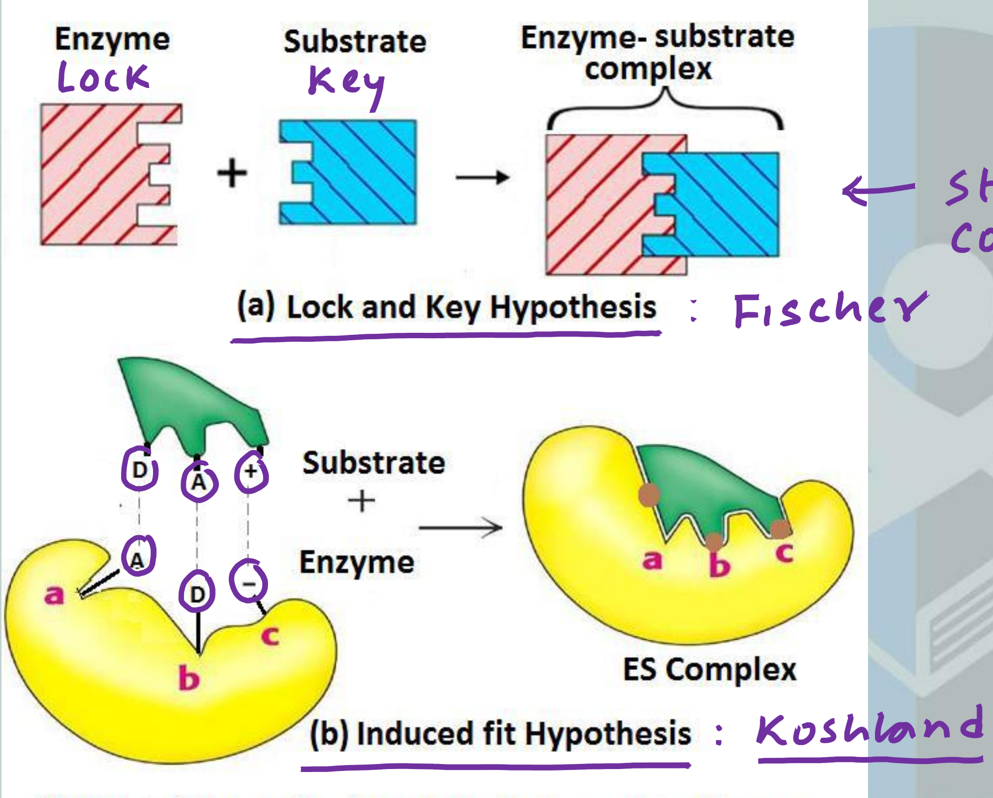



Figure 3: Interaction between enzyme and substrate

**Non-covalent Interactions between enzyme and substrate**

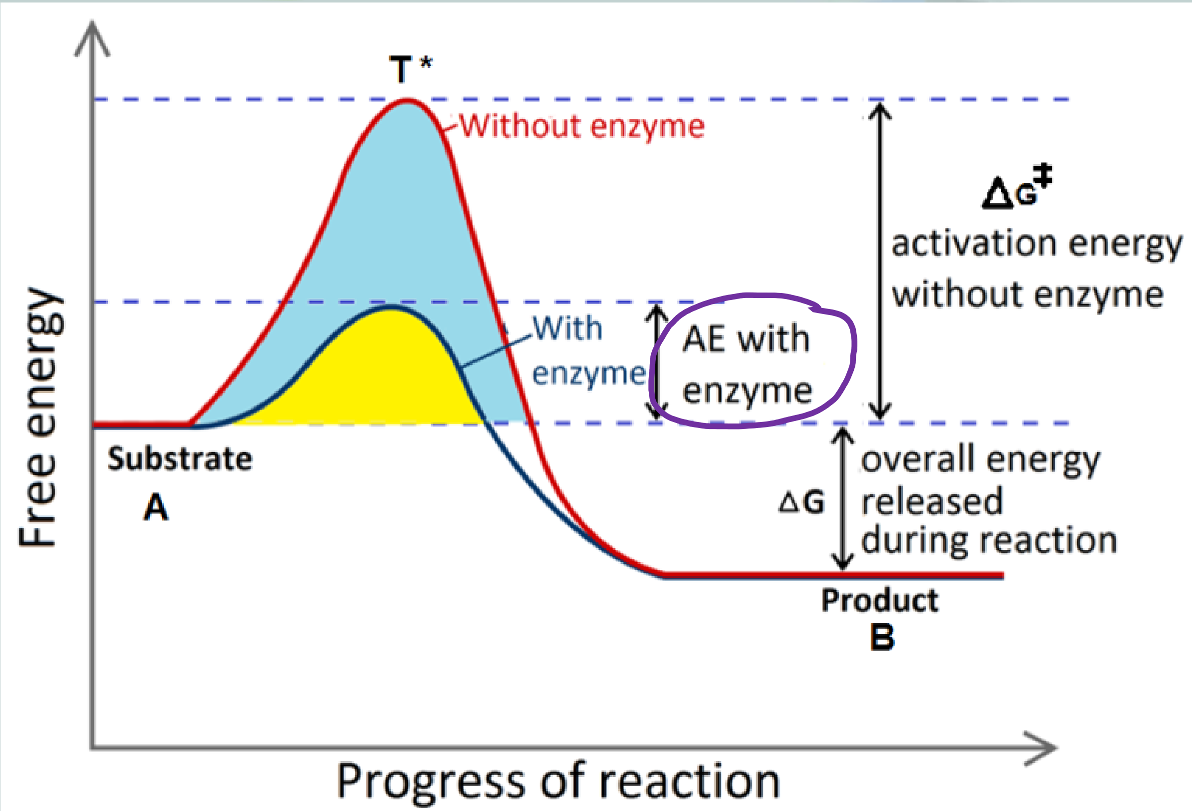
↓

Specific

↓

Induces conformational change such that substrate can get fit into it.

## Free energy of activation

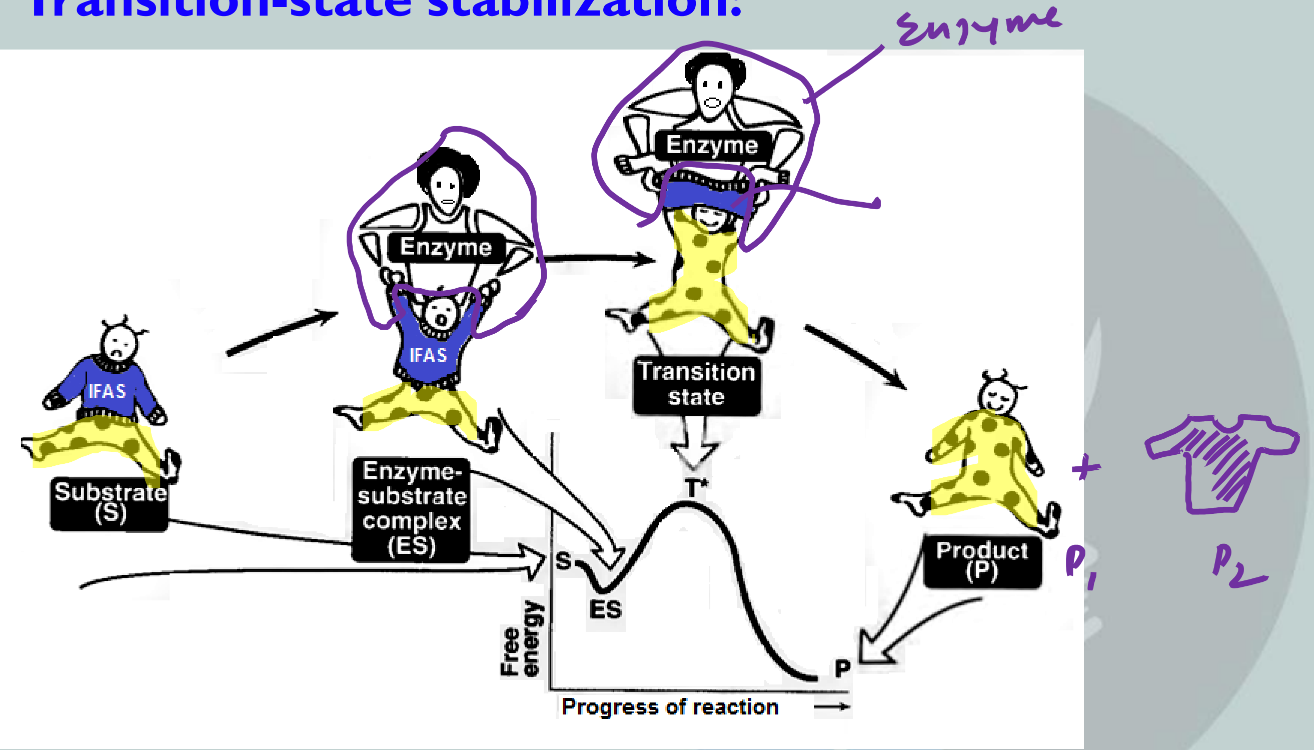



- Enzyme provide alternative pathway for reaction completion

Needs lesser activation energy

Rate increases

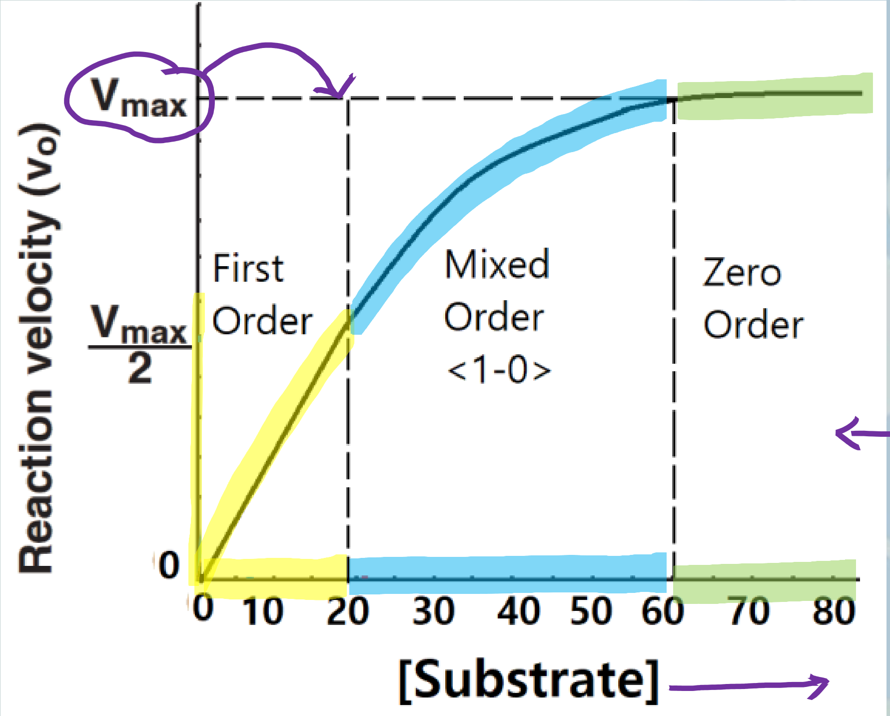
# Transition-state stabilization:




Binding energy due to multiple weak interactions with substrate, decreases activation energy required to achieve transition state

## FACTORS AFFECTING REACTION VELOCITY

# A. Substrate concentration



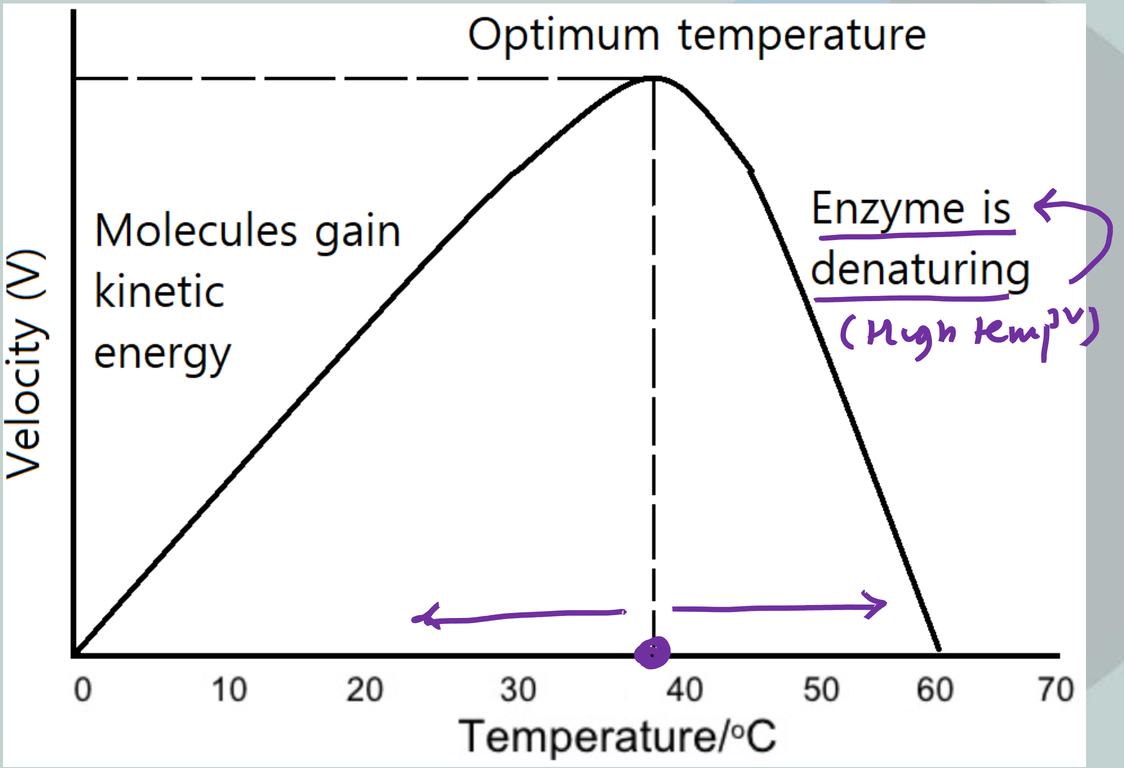

low [S] = First order

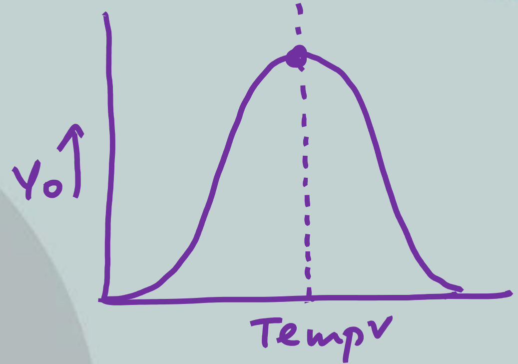
Very high [S] = zero order

Intermediate [S] concⁿ
= mixed order
= <1 – 0>

Rectangular Hyperbolic curve.

## B. Temperature



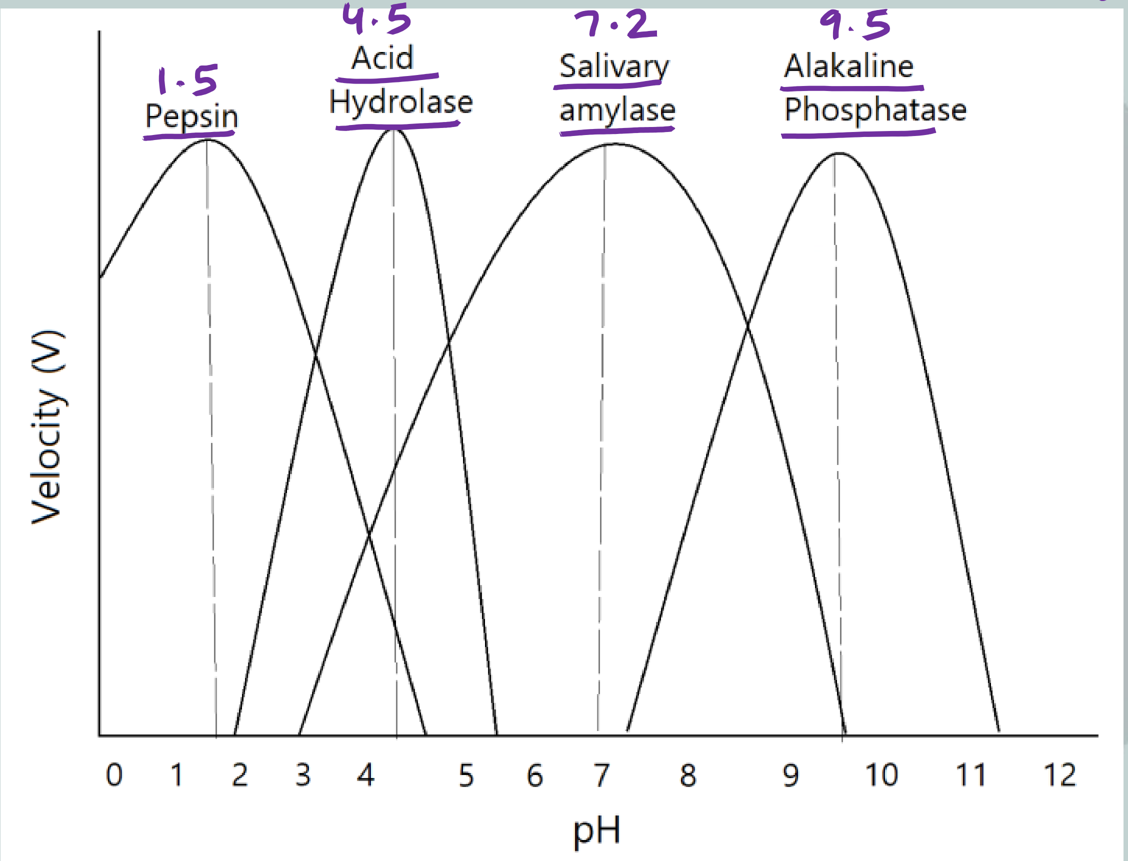



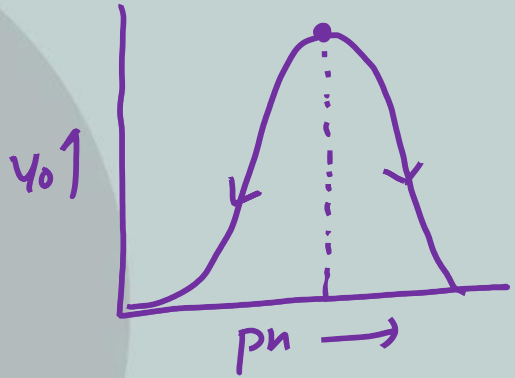

Low temp^r: [S] has less Kinetic energy

High temp^r: Enzyme denaturate

optimum temp^r = Best activity

**C. pH** → change in ionization state of protein which may alter active conformation



## Enzyme Classes: International Union of Biochemistry and Molecular Biology (IUMB)

O T H L I Ligase
1 2 3 4 5 6

| Group | Class of enzyme | Reaction carried out | | Example |
|---|---|---|---|---|
| 1 | Oxidoreductases | Transfer of electrons | $A^- + B \rightarrow A + B^-$ | Alcohol dehydrogenase<br>Oxidase, Reductase |
| 2 | Transferases | Transfer of C-, N- or P-containing groups | $A\text{-}B + C \rightarrow A + B\text{-}C$ | Hexokinase (Phosphate) |
| 3 | Hydrolases | Bond cleavage by addition of water | $A\text{–}B + H_2O \rightarrow A\text{-}H + B\text{-}OH$ | Trypsin<br>Digestive enzyme |
| 4 | Lyases<br>(Synthase) | Cleavage of C–C, C–S and C–N bonds often to form a double bond | A—B (with C and D attached below) $\leftrightarrow$ $\boxed{A{=}B}$ + C–D | Pyruvate decarboxylase<br>RUBISCO, Aldolase<br>Citrate lyase<br>malate synthase |
| 5 | Isomerases | Formation of optical or geometric isomers | A—B (C, D below) $\rightarrow$ A—B (D, C below) | • Maleate isomerase<br>• Epimerase<br>• Racemase<br>• mutase |
| 6 | Ligases<br>(Synthetase) | Hydrolysis of high energy phosphates to form new bonds. | $A + B \rightarrow A\text{–}B$<br>ATP (GTP) → ADP (GDP) | • Pyruvate carboxylase<br>• DNA ligase |

Cofactor

Inorganic Metals

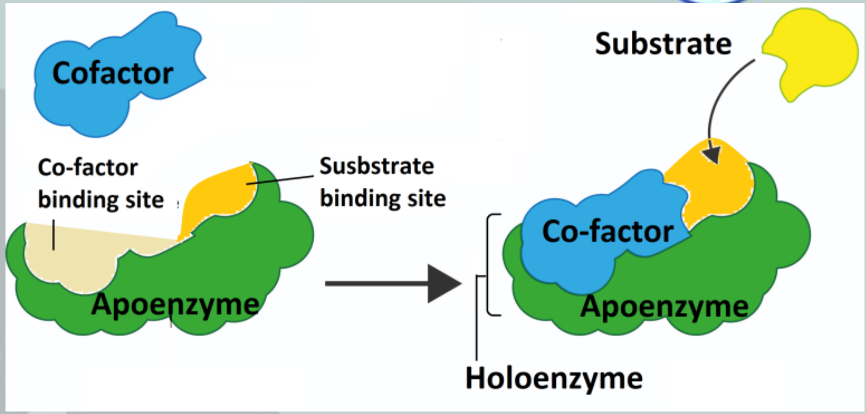
Organic Co-enzymes

Weak binding

Non-covalent

Strong binding Prosthetic group

covalent



# Inorganic Co-factors

| | |
|---|---|
| $Zn^{2+}$ | Carbonic anhydrase<br>Carboxypeptidase<br>Alchohol dehyrogenase |
| $Mg^{2+}$<br>$Mg^{2+}$ | Restriction endonuclease<br>DNA/RNA polymerase<br>Hexokinase |
| $Ni^{2+}$ | Urease |
| Mo | Nitrate reductase<br>Nitrogenase |
| Se | Glutathione peroxidase |
| $Mn^{2+}$ | Superoxide dismutase<br>Water splitting enzyme (PSII)<br>Arginase |
| $K^{+}$ | Propionyl CoA carboxylase |
| $Cu^{2+}$ | Tyrosinase<br>Uric acid oxidase<br>Ascorbinc acid oxidase<br>Cytochrome oxidase |
| $Fe^{2+}$ | Cytochrome<br>Catalase<br>Hydrogenase<br>Nitrogenase |

Divalent metal ions

$Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Mo^{2+}$, $Mn^{2+}$

$K^{+}$ : Pyruvate kinase

monovalent Propionyl CoA Carboxylase

## Co-enzyme: carriers of specific chemical groups

, mostly derived from water soluble vitamins (B & C)

| Organic co-factor | | |
|---|---|---|
| Thiamine pyrophosphate ✓ B1 | Transketolase<br>Pyruvate dehydrogenase | C-C cleavage & formation $rx^n$ |
| B2 Flavin mono nucleotide (FMN) | NADH dehyrogenase | electron transfer (oxd-Red) |
| Flavin adenine nucleotide (FAD) | Succinate dehyrogenase | |
| B3 Nicotinamide adenine dinucleotide (NAD) | Lactate dehydrogenase | electron transfer (oxd-Red) |
| B6 Pyridoxal phosphate (PLP) | ✓ Glycogen phosphorylase<br>✓ Amino trnasferase | Amino group transfer |
| B5 Coenzyme A (CoA) | Acetyl CoA carboxylase | Acyl group transfer |
| Biotin B7 | Pyruvate carboxylase | carboxylation $rx^n$ |
| B12 5'-Deoxyadenosyl cobalamin | Methylmalonyl mutase | methyl group (1C) transfer |
| B9 Tetrahydrofolate (THF) | Thymidylate synthase | 1C, methyl or formyl group transfer |
| C Ascorbic acid | Prolyl hydroxylase | OH group transfer |
| Menaquinone (vit K) | γ- Carboxylase | $CO_2$ transfer |
| S-adenosyl methionine (SAM) | DNA methyl transferase | methyl transfer |
| Glutathione E-C-G | Glutathione peroxidase | oxidation $rx^n$. |
| Lipoamide | Pyrvuate dehydrogenase | |
| Heme | Cytochrome, Catalase | |

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